Pestic. Sci. 1997, **50**, 151–155

Extended Summaries
Pesticides in Food and Drink

The following are extended summaries based on papers presented at the 1st European Pesticide Residue Workshop, 'Pesticides in Food and Drink', held at Alkmaar, The Netherlands on 10–12 June, 1996. They are entirely the responsibility of the authors and do not necessarily reflect the views of the Editorial Board of Pesticide Science.

Harmonization of Pesticide Residue Analytical Methods by the Technical Committee 275 of the Comité Européen de Normalisation (CEN/TC 275)

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Introduction

Standardization is a useful tool for ensuring compatibility between products or between appropriate levels of their quality. This applies also to the test methods needed to establish the conformity desired. Up to now, standards have been set by national standards bodies, sometimes very differently from one country to another and sometimes in an equivalent manner thanks to international cooperation, notably within the framework of ISO (International Organization for Standardization). A major proportion of these national documents are now being replaced gradually by a single set of European Standards. CEN (Comité Européen de Normalisation), the organization responsible for planning, drafting and adopting these standards, is an association of the national standards bodies of 18 European Union and EFTA states, incorporated in Brussels under Belgian law. Several international organizations have a liaison status in order to support the mutual exhange of information.

In 1990, the CEN Technical Bureau decided to establish a Technical Committee No. 275 (TC 275) on 'Food analysis, horizontal methods'. For the time being, its scope is restricted to 'Additives, residues and contaminates in foods'. The secretariat of this Technical Committee is situated at DIN (Deutsches Institut für Normung) in Germany. Since its first meeting in 1991, TC 275 has formed nine working groups (WG). Among them are WG 3 on 'Pesticides and polychlorinated biphenyls in fatty foods' and WG 4 on 'Pesticides in non fatty foods'. The convener and the secretariat are provided by DIN and AFNOR (Association Française de Normalisation), respectively.

TC 275 has asked all working groups to identify the following: (1) which successfully collaboratively tested methods are available; (2) which of these give comparable results, and (3) which of the available methods, if possible one of those which give comparable results, is the most appropriate for adoption as a reference method. In addition, the methods selected should be suitable for official food inspection as well as for quality control in the food industry. Each national standard body was invited to send up to three experts to meetings of each working group, but in most cases, the meetings of WG 3 and WG 4 were attended by only some 15 delegates. A close cooperation of the groups was made easy by numerous dual memberships.

Both WGs concentrated first on those multi-residue methods which cover a broad range of pesticides in a great variety of substrates and which are of primary importance in analytical practice. The substrates for WG 3 were all the fat-containing products of animal origin based on fish, meat, milk and eggs which may contain residues of non-polar and persistent organochlorine pesticides (OCs) and of polychlorinated biphenyl (PCB) congeners. WG 4, on the other hand, dealt mainly with fruits and vegetables and therefore with the whole pattern of insecticides, fungicides, etc., which are applied in agricultural practice.

Problems of standardization

An inquiry among all CEN member bodies resulted in a large diversity of submitted methods, which reflects the

 Pestic. Sci. 0031-613X/97/$17.50. Printed in Great Britain

various national preferences after some three decades of experience. Considering the different ways for extracting the substrate, and in particular for the clean-up, the experts of both WGs felt unable to identify one of the proposed procedures as the prime method to be adopted as a reference. Instead they decided unanimously to compile in the standards those procedures which are validated and are widely used throughout Europe. They were all classified as possessing an equal status.

The arguments for that classification result from long-standing experience and many collaborative studies. First, the quality of an analytical result depends much more on the expertise and training of the analyst than on the suitability of the analytical method used. In other words, a well-trained analyst will supply good results even if he uses a less appropriate procedure, whereas an excellent procedure in the hands of a less competent analyst gives no guarantee whatsoever of a fair result. Second, many years' experience was available in each country for the particular procedure it had submitted. Without doubt, an analyst who is well acquainted with the advantages and disadvantages of his usual procedure will supply the best results with just this procedure and not with a novel procedure which he would be obliged to use as the consequence of a rigid standardization.

Another problem was the status of the final determination of the residues by gas chromatography (GC) in a standard. First, there is a wide range of GC columns and of specific detectors in current use and the GC conditions must often by adjusted to the actual problem. Second, the quality of an analytical result depends to a high degree on the actual efficiency of the GC system, which may differ even from one hour to another; any pollution may seriously affect the separation efficiency, the detector response, or both. For this reason, the decision was to exclude the GC conditions from the proper standard text and to give only some informative guidance. Consequently, the standards apply only to the extraction and clean-up procedures.

A third problem arose, because TC 275 had defined some essential criteria as a basis for selecting the appropriate methods from the submitted methods. One of them requires verification of the performance of a method by a collaborative trial according to ISO 5725. In pesticide multi-residue analysis, however, this cannot be accomplished. The broader the applicability of a procedure, the larger is the number of the possible pesticide/substrate combinations. Validating all of them would involve an enormous expenditure with hundreds of collaborative studies.

The second objection derives from the very low concentrations of the pesticides to be determined. In such a trace analysis, the test results under reproducible conditions vary considerably, e.g. by 100% at the 0.01 mg kg^{-1} level. Indeed, a lot of collaborative studies have been performed up to now, but all attempts to calculate the repeatability and the reproducibility in a statistically correct way as required by ISO 5725 have failed. In the meantime, TC 275 has agreed that this criterion is not necessarily valid for pesticide multi-residue analysis.

When summarizing all the points discussed above, it becomes evident that pesticide multi-residue analysis cannot be subjected to standardization in its conventional meaning. However, what the WGs have succeeded in is to realize that there is a very valuable harmonization of methods across Europe. As a consequence, the result from a pesticide residue analysis obtained in one European country is now much more easily approved in another country, if the analytical method used is adopted as a European Standard. This has to be considered as a real progress in European cooperation as far as methodology is concerned. But one should bear in mind that a validated method does not necessarily guarantee a valid result, for this will depend much more on the experience and the actual performance of the analyst.

Multi-residue methods for fatty foods

WG 3 has elaborated a final draft for the provisional standard prEN 1528 on 'Fatty food—Determination of pesticides and polychlorinated biphenyls (PCBs)'.[1] It is subdivided into four parts and is now on its way to the CEN authorities for launching the formal vote for its acceptance.

Part 1, dealing with 'General considerations,' describes the scope of the standard and general aspects with regard to reagents, apparatus, determination, precision, the routine practical limit of determination, etc. In this context, a difference of 0.01 mg kg^{-1} between test results at the 0.01 mg kg^{-1} level is considered acceptable under reproducibility conditions. Part 2, 'Extraction of fat, pesticides and PCBs, and determination of fat content' lists several procedures for extracting the fat components containing the residues from different groups of fat-containing foodstuffs. Part 3, 'Clean-up methods,' presents the details of eight selected procedures for the clean-up of fats and oils or the isolated fat portion respectively, using techniques such as liquid/liquid partition, adsorption column chromatography or gel permeation chromatography. Finally, Part 4 on 'Determination, confirmatory tests, miscellaneous' gives guidance on some recommended conditions for the use of gas-chromatographic columns and detectors, a general survey on confirmatory techniques and the details of an additional clean-up procedure for large quantities of fats using Calflo E.

The clean-up methods included in Part 3 are given in Table 1. Method A is the classic AOAC procedure[2] which set the fashion in the early days of OC analysis. Method B, according to Specht[3] is rather similar but

TABLE 1
Clean-up Methods for Fats and Oils or the Isolated Fat Portion as Compiled in Part 3 of prEN 1528

Letter	*Title*	*Reference*
A	Liquid-liquid partitioning with acetonitrile and clean-up on a Florisil column (AOAC)	2
B	Liquid-liquid partitioning with dimethylformamide and clean-up on a Florisil column (Specht)	3
C	Column chromatography on activated Florisil (AOAC)	4
D	Column chromatography on partially deactivated Florisil (Stijve)	5
E	Column chromatography on partially deactivated aluminium oxide (Greve & Grevenstuk)	6
F	Gel permeation chromatography (GPC) (AOAC)	7
G	Gel permeation chromatography (GPC) and column chromatography on partially deactivated silica gel (Specht)	8
H	High performance gel permeation chromatography (HPGPC) (MAFF)	9

uses dimethylformamide for partitioning and Florisil deactivated by 5% water. Method C[4] has been designed for some OCs and PCB congeners in fish samples, but will also be applicable to other substrates. Whereas these three methods use for elution mixtures of petroleum distillate with a low or medium percentage of diethyl ether, method D according to Stijve[5] uses Florisil (+3% water) along with petroleum distillate + dichloromethane (4 + 1 by volume). A small-scale procedure for this technique is also included. WG 3 is aware that Florisil is a very crucial adsorbent, for the activity may vary from one batch to another, and appropriate precautions have to be taken. Basic aluminium oxide (9% water) is an alternative as used in Method E according to Greve and Grevenstuk.[6]

The most promising technique is gel permeation chromatography (GPC) which is expected to replace the Florisil-based procedures in the future. For a Bio Beads GPC column, Method F from AOAC[7] uses cyclohexane + dichloromethane (1 + 1 by volume). Method G according to Specht[8] manages the elution without use of a chlorinated solvent by using cyclohexane + ethyl acetate (1 + 1 by volume). In addition, a miniaturized column of partially deactivated silica gel provides a further clean-up and fractionates the residues according to their polarity, thus yielding extra information for their identification. This is particularly useful for separating the residues of OC pesticides and of PCB congeners. Method H, submitted by the UK MAFF,[9] provides a high-performance GPC separation. At its 1995 Meeting, the Technical Committee has accepted the provisional standard prEN 1528 and has submitted it to the CEN authorities and member bodies prior to launching the formal vote for its acceptance.

Multi-residue methods for non-fatty foods

The simultaneous efforts of WG 4 on pesticide residues in non-fatty foods resulted in the working document[10] for a provisional standard prEN 12393, which consists of three parts. Since WG 3 and WG 4 wanted their standards to be as similar as possible, Part 1 on 'General considerations' differs from Part 1 of prEN 1528 only in some details, e.g. on sampling and the preparation of the test sample and the test portion. In the same way, Part 3 on 'Determination and confirmatory tests' is restricted to some guidance on suitable GC conditions and techniques for confirming the results.

The 'Selected methods for extraction and clean-up' compiled in Part 2 are given in Table 2. Most of them use acetone for extraction and a subsequent partitioning step after dilution with water except for Method P. Sweep co-distillation has been included in Method L,[11] but it will probably be superseded in future by other procedures. Central to Method M[12] is an adsorption column with a mixture of charcoal and silica gel which is very efficient for removing plant co-extractives. Method N,[13] using a column with four adsorbents first appeared in the late 1970s in a draft of an official EEC document, but there is little available experience of its use. Method O is essentially the Luke method,[14] if the crude extracts obtained merely from partitioning steps are directly injected into a gas chromatograph equipped with very selective detectors. For further clean-up, a Florisil column is used under the usual AOAC conditions, either with petroleum distillate + diethyl ether mixtures or alternatively with mixtures of petroleum distillate and dichloromethane containing small percentages of acetonitrile. In general, acetonitrile yields less co-extractives than acetone, but it is found only as part of the classic AOAC procedure as Method Q.[2]

Just as for fatty foods, GPC is on the way to take over the premier position for the clean-up. A very broad range of applicability has been proved for Method P according to Specht.[8] The GPC conditions and the supplemental small silica gel column are nearly the same as in Method G. They are preceded here by acetone extraction at a defined water content and partition into

TABLE 2
Methods for Extraction and Clean-up of Non-fatty Foods as Compiled in Part 2 of prEN 12393

Letter	*Title*	*Reference*
L	Extraction with acetone and liquid-liquid partitioning with dichloromethane and clean-up by sweep co-distillation	11
M	Extraction with acetone, liquid-liquid partitioning with dichloromethane and clean -up on a silica gel/charcoal column	
N	Extraction with acetone and liquid-liquid partitioning with dichloromethane and clean-up on a mixture of Florisil, Celite, Attaclay, charcoal column	13
O	Extraction with acetone, liquid-liquid partitioning with dichloromethane/light petroleum distillate, if necessary clean-up on Florisil	
P	Extraction with acetone, liquid-liquid partitioning with dichloromethane and clean-up with gel permeation and silica gel chromatography	
Q	Extraction with acetonitrile, liquid-liquid partitioning with light petroleum distillate and clean-up on a Florisil column	2
R	Extraction with ethyl acetate and evaporation, if necessary clean-up with gel permeation chromatography	15

dichloromethane without addition of water. Method R[15] applies the same GPC conditions, but the extraction is performed with ethyl acetate, which has been shown to be equivalent to water-miscible solvents. In this case, no partitioning step is required.

Again in prEN 12393, all methods included have an equal status, no prime method has been identified, and a rigid standardization has been avoided, since it is neither realistic nor desirable in pesticide multi-residue analysis. However, a valuable harmonization was achieved again in the sense that an analyst using one of the selected methods can be confident of applying a technique which international expertise has adopted as reliable and which facilitates the acceptance of the results in another European country. Another argument for simply harmonizing the methods is that residue analysts always like to introduce some minor or major individual modifications for avoiding interference caused by co-extractives. This is particularly true for plant material with its wide range of different components. Such modifications, however, must not be carried so far that complete steps from different methods are exchanged. For this reason, prEN 1293 states explicitly that 'it is possible to choose one method, but then necessary to follow the whole method once selected.'

Further activities

With the completion of prEN 1528 on fatty foods, WG 3 has in principle performed its task for the five years, until a review of every standard has to take place. But because the methods included in the standard are continuously developing, WG 3 has decided to be concerned with the review already at an early stage. In a final resolution, the members stressed the need to collect methods and techniques currently in use which are not included in the standard at this stage. In addition, they made two further points: All CEN member countries are requested to encourage the validation of methods by appropriate collaborative studies and they are invited to comment on the application of the methods included in the present standard. WG 4 will probably proceed in a similar way.

In the meantime, work is going on in WG 4 for other groups of pesticides. For instance, the working document for prEN 12396[16] has been completed on 'Quantitative determination of dithiocarbamate residues and thiuram disulfide fungicides by evolution of carbon disulfide'. It comprises two procedures for determining the carbon disulfide which is released from the compounds by concentrated hydrochloric acid. Part 1 is based on the well-known formation of a yellow copper complex with diethanolamine, and its photometric determination.[17] Part 2 deals with the head-space gas chromatography of the carbon disulfide using electron-capture or flame-photometric detection.[18] Both parts have already been accepted by TC 275 and are on their way to the CEN enquiry for technical comments. For the time being, work on Part 3 is in progress which uses the formation of xanthogenate and its photometric determination in the UV range. This method is particularly sensitive and will be suitable also for the low maximum residue levels which have been set up very recently.

Another subject of WG 4 has been the inorganic bromide which is formed as a residue from bromine-containing fumigants. The drafted standard contains the derivatization of bromide with ethylene oxide or propylene oxide and gas chromatography with electron-capture detection.[19] As an alternative, a second procedure uses an ashing procedure prior to derivatization.[20] In this way, small portions of organic bromine which may still be present in the sample are also covered by the determination.

In the near future, WG 4 will also deal with methyl carbamate insecticides, with fungicides of the benzimid-

azole type and with maleic hydrazide residues. For all these compounds, WG 4 will do its very best to select validated methods which are suitable for inclusion into additional European standards.

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